Data Evaluation Report on the Acute Dietary Toxicity of DPX-MAT28 Technical (Aminocyclopyrachlor) to Northern Bobwhite Quail (*Colinus virginianus*)

PMRA Submission Number {.....} EPA MRID Number 47560120

Data Requirement:

PMRA Data Code	{.....}
EPA DP Barcode	358148
OECD Data Point	{.....}
EPA MRID	47560120
EPA Guideline	850.2200

Test material: DPX-MAT28 Technical **Purity:** 92.2%
Common name Aminocyclopyrachlor
Chemical name:
IUPAC: 6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid
CAS: 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
CAS No.: 858956-08-8
Synonyms: None reported

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Staff Scientist, Dynamac Corporation
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Date: 07/13/09

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Date: 10/05/09

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category	{.....}	[For PMRA]
EPA PC Code	None	

Date Evaluation Completed: 10/05/09

CITATION: Hubbard, P.M., K.H. Martin, and J.B. Beavers. 2007. DPX-MAT28 Technical: A Dietary LC_{50} Study with the Northern Bobwhite. Unpublished study performed by Wildlife International Ltd., Easton, MD. Laboratory Project No. 112-597. Study sponsored by E.I. du Pont de Nemours and Company, Wilmington, DE. Study initiated March 28, 2007 and submitted June 6, 2007.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute dietary toxicity of a pesticide to avian species. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The acute dietary toxicity of DPX-MAT28 Technical (aminocyclopyrachlor) to 10-day old Northern bobwhite quail (*Colinus virginianus*) was assessed over 8 days. DPX-MAT28 was administered to the birds in the diet at nominal concentrations of 0 (negative control), 562, 1000, 1780, 3160, and 5620 mg ai/kg diet. Mean-measured concentrations were <25.0 (<LOD, control), 555, 982, 1790, 3200, and 5290 mg ai/kg diet, respectively. No treatment-related mortality, clinical signs of toxicity, or effects on body weight or food consumption were indicated during the study. The 8-day acute dietary LC_{50} was >5290 mg ai/kg diet, and the NOAEC was 5290 mg ai/kg diet. DPX-MAT28 Technical (aminocyclopyrachlor) would be classified as practically non-toxic to juvenile Northern bobwhite quail (*Colinus virginianus*) in accordance with the classification system of the U.S. EPA.

This toxicity study is scientifically sound and classified as acceptable and, thus, satisfies the guideline requirement for an acute dietary toxicity study for the bobwhite quail.

Results Synopsis

Test Organism Size/Age(Mean Weight): 10-days old; 18 to 25 g

LC_{50}: >5290 mg ai/kg diet 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

NOAEC: 5290 mg ai/kg diet
LOAEC: >5290 mg ai/kg diet

Endpoints affected: none

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: U.S. EPA Ecological OPPTS No. 850.2200 (1996)
U.S. EPA Pesticide Assessment Guidelines, §71-2 (1982)
ASTM Standard E857-87 (1987)
OECD Guideline No. 205 (1984)

Deviations from OPPTS Guideline No. 850.2200 included:

1. The pre-test health of the population (including mortality) was not reported. Birds should not be used if >5% of the total test population die during the 72-hour period preceding the test.
2. It was unclear if a brooder temperature gradient (of approximately 22 to 38°C) was provided for the ducklings.
3. The photoperiod (16 h light:8 h dark) was slightly different than recommended (14 hr light:10 hr dark).

These deviations do not affect the scientific soundness or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with the GLP as published in 40 CFR Part 160 with the following exception: periodic analyses of feed and water for potential contaminants were not conducted in accordance with GLP, but were performed using a certified laboratory and standard U.S. EPA analytical methods.

A. MATERIALS:

1. Test Material DPX-MAT28 Technical (aminocyclopyrachlor)

Description: Solid

Lot No./Batch No.: Not reported; Reference No. DuPont-21466

Purity: 92.2%

Stability of compound under test conditions: The 5-day stability of DPX-MAT28 Technical was determined under actual use conditions. Recoveries averaged 103 to 114% of initial measured concentrations.

Storage conditions of test chemicals: Ambient conditions

Physicochemical properties of Aminocyclopyrachlor.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	

Parameter	Values	Comments
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species (common and scientific names): Northern bobwhite quail (*Colinus virginianus*)
(EPA recommends using either bobwhite quail or mallard duck.)

Age at study initiation: 10 days old
(EPA recommends: 10-14 days old)

Weight at study initiation (mean and range): 18 to 25 g

Source: Wildlife International production flock

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: None reported. The dietary concentrations were established based upon known toxicity data.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period:	10 days	All birds were observed daily during acclimation, and any birds exhibiting abnormal behavior or physical injury were not used for the study.
Conditions: (same as test or not)	Same as test	
Feeding:	Game bird ration formulated to Wildlife International Ltd.'s specifications and water from the town of Easton public water supply were offered *ad libitum*.	
Health: (any mortality observed)	Not reported.	

Parameter	Details	Remarks *Criteria*
Pen size and construction materials	Wire mesh and/or galvanized sheeting cages measuring 72 x 90 x 23 cm.	Birds were housed in groups of five. The floor area was 1296 cm^2 per bird. *Recommended pen size is about 35 x 100 x 24 cm*
Test duration	5 days with treated feed followed by 3 days with untreated feed	*Recommended test duration is 5 days with treated feed and at least 3 days observation with "clean" feed.*
Test concentrations nominal: measured:	0 (negative control), 562, 1000, 1780, 3160, and 5620 mg/kg diet <25 (<LOD, control), 555, 982, 1790, 3200, and 5290 mg ai/kg diet, respectively	Dietary concentrations were adjusted for the purity of the test substance. Samples were collected for analysis on Day 0. Mean-measured concentrations ranged from 94 to 101% of nominal. *Five or six test concentrations should be used in a geometric scale, unless the LC_{50} > 5000 mg ai/kg diet.*
Solvent/vehicle, if used type: amount:	N/A	*Recommended solvents include distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. The solvent should not be more than 2%.*
Diet preparation and feeding	The appropriate amount of test substance was blended for approximately 1 minute with a small portion of basal diet. This mixture was then quantitatively combined with the remaining basal diet and mixed for a total of 20 minutes using a Hobart mixer.	Diets were prepared on the day of test initiation and presented to the birds. *The control group should be tested with a diet containing the maximum amount of vehicle used in treated diets.*
Feed withholding period	None	
Stability and homogeneity of test material in the diet determined (Yes/No)	Stability – yes Homogeneity – yes	

Parameter	Details	Remarks *Criteria*
Number of birds per replicate/groups for negative control: for vehicle control: for treated:	 5 birds/cage N/A 5 birds/cage	*The recommended number of birds per replicate is a minimum of ten.*
Number of replicates/group (if used) for negative control: for vehicle control: for treated:	 6 replicate cages N/A 2 replicate cages/level	There were a total of 30 control birds and 10 treated birds/level, fulfilling OPPTS guidelines.
Test conditions temperature: relative humidity(%): photoperiod:	 Brooder: *ca.* 39°C Room: 28.6 ± 1.0°C Room: 21 ± 8% 16 hours light/8 hours dark	It is recommended that a temperature gradient in the pen of approximately 22 to 38°C is provided to allow young birds to seek a proper temperature. The photoperiod slightly exceeded recommendations (14 hours light/10 hours dark); light intensity was approximately 181 lux. ***Recommended brooder temperature is** about 35°C (95°F)* ***Recommended room temperature is** 22-27°C (71-81°F)* ***Recommended relative humidity is** 30-80%* ***Recommended photoperiod** is14 hours of light/10 hours dark.*
Reference chemical, if used	N/A	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks
Parameters measured (mortality/body weight/ mean feed consumption/ others)	- Mortality - Clinical signs of toxicity - Body weight - Food consumption	

Parameters	Details	Remarks
Indicate the stability and homogeneity of test chemical in the diet	Stability: Stability was assessed at all levels after 5 days under actual use conditions. Recoveries averaged 103 to 114% of initial measured concentrations. Homogeneity: Homogeneity was assessed by collecting samples from the top, middle, and bottom left and right areas (six samples) from treated feed prepared at 562 (low) and 5620 (high) mg/kg diet. Coefficients of variation were 1.68 and 2.11%, respectively.	
Indicate if the test material was regurgitated	No regurgitation was indicated.	
Treatments on which necropsies were performed	None performed	As no mortalities were observed, gross necropsies were not necessary.
Observation intervals	Birds were observed at least twice daily for mortality and clinical signs of toxicity. Body weights were determined on Days 0, 5, and 8. Feed consumption was calculated each day during the exposure period and once during the recovery period (Days 6 to 8).	
Were raw data included?	Raw body weight and food consumption data were not provided.	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortality was observed in the control or any treatment group. The 8-day acute dietary LC_{50} was >5620 mg ai/kg diet. The NOAEC for mortality was 5620 mg ai/kg diet.

Table 3: Effect of DPX-MAT28 Technical (Aminocyclopyrachlor) on Mortality of Bobwhite Quail.

Treatment, mg ai/kg diet Mean-measured (and nominal) conc.		No. of birds per treatment	Cumulative mortality				
			day 1	day 2	day 3	day 4	day 8
Control		30	0	0	0	0	0
555 (562)		10	0	0	0	0	0
982 (1000)		10	0	0	0	0	0
1790 (1780)		10	0	0	0	0	0
3200 (3160)		10	0	0	0	0	0
5290 (5620)		10	0	0	0	0	0
NOAEC		5620 mg ai/kg diet (nominal)					
LC_{50}		>5620 mg ai/kg diet (nominal)					
Reference chemical	mortality	N/A					
	LC_{50}	N/A					
	NOAEC	N/A					

B. SUB-LETHAL TOXICITY ENDPOINTS:

There were no overt signs of toxicity at any of the concentrations tested. Two control birds were observed limping, presumably due to injury, from Days 5 to 8 of the study. One bird from the 562 mg ai/kg diet level was also observed limping on Day 1, but had recovered by Day 2. All remaining birds (all levels) were normal in appearance and behavior throughout the test. The NOAEC for clinical signs of toxicity was 5620 mg ai/kg diet.

Visual inspection of the data indicated no apparent treatment-related effects upon body weight or food consumption at any treatment level. The NOAEC values for body weight and feed consumption data were 5620 mg ai/kg diet.

Using body weight and food consumption data, daily dietary doses were estimated to be 129, 212, 389, 719, and 1177 mg ai/kg/day for the 562, 1000, 1780, 3160, and 5620 mg ai/kg diet levels, respectively.

Table 4: Sublethal Effect of DPX-MAT28 Technical (Aminocyclopyrachlor) on Bobwhite Quail.

Treatment, mg ai/kg diet Mean-measured (and nominal) conc.	Observation									
	Body weight change, g			Food consumption, g/bird/day						
	Days									
	0-5	5-8	0-8	0-1	1-2	2-3	3-4	4-5	0-5	6-8
Control	12	8	20	12	6	6	5	7	7	8
555 (562)	13	8	21	11	5	5	5	6	6	9
982 (1000)	12	8	20	11	4	5	4	5	6	8
1790 (1780)	12	9	21	10	5	5	4	5	6	9
3200 (3160)	13	8	21	10	5	5	5	6	6	9
5290 (5620)	13	8	21	10	5	5	4	5	6	9
NOAEC	5620 mg ai/kg diet (nominal)			5620 mg ai/kg diet (nominal)						
EC_{50}	>5620 mg ai/kg diet (nominal)			>5620 mg ai/kg diet (nominal)						
Reference chemical NOAEC	N/A			N/A						
Reference chemical EC_{50}	N/A			N/A						

C. REPORTED STATISTICS:

As no mortalities were observed, the 8-day LC_{50} was visually determined to be greater than the highest concentration level. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Statistical analyses were not required to verify the results of this study. There was no mortality and body weight was clearly not affected by treatment in any dose-dependent manner. Food consumption was lower at higher levels between days 0 and 1 (probably due to taste aversion), but these differences were not maintained beyond this time period and they had no effect on body weight.

LC_{50}: >5290 mg ai/kg diet 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 5290 mg ai/kg diet

Endpoints affected: none

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA OPPTS Guideline No. 850.2200 affecting the scientific soundness or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with those of the study authors. Results were provided in terms of mean-measured concentrations in the Executive Summary and Conclusions sections of the DER.

Portions of treated feed (10.0 g) were extracted with 100 mL of methanol:water (80:20, v:v) by sonicating for 15 minutes followed by shaking at *ca.* 250 rpm for *ca.* 60 minutes. Aliquots were centrifuged at *ca.* 1500 rpm for *ca.* 10 minutes, diluted as needed with methanol:water (80:20, v:v), and analyzed for aminocyclopyrachlor using HPLC with UV (240 nm) detection. The analytical LOD and LOQ were 25.0 and 50.0 mg ai/kg diet, respectively.

Avian diet was fortified at 50.0 or 6000 mg ai/kg and analyzed concurrently with the Day 0 and 5 samples. Mean recoveries were 93% for both days. Test sample results were not corrected for the mean procedural recoveries from each sample set.

Experiment study dates were March 29 to April 6, 2007.

G. CONCLUSIONS:

This study is scientifically sound and classified as acceptable. No treatment-related mortality, clinical signs of toxicity, or effects on body weight or feed consumption were observed at any test level following a 5-day acute dietary administration of DPX-MAT28 Technical (aminocyclopyrachlor) at up to the limit concentration, followed by a 3-day recovery period. The LC_{50} was >5290 mg ai/kg bw (>limit concentration), and the NOAEC was 5290 mg ai/kg bw.

LC_{50}: >5290 mg ai/kg diet 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

NOAEC: 5290 mg ai/kg diet
LOAEC: >5290 mg ai/kg diet

Endpoints affected: none

III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.2200: *Avian Dietary Toxicity Test.*

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms,* Subsection 71-2, Environmental Protection Agency, Office of Pesticide Programs, Washington, D.C.

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